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## About the Kits

### Description

Novagen's Competent Cells enable convenient, efficient construction of plasmid recombinants. The cells are grown and made chemically competent by an optimized procedure, followed by verification of cloning efficiency and strain identity. The cells in the standard kits are provided as frozen 0.2 ml aliquots; each vial can be used for 10 transformations. The cells in the Singles™ kits are provided as single-use 50 µl aliquots for greater efficiency and convenience, and are packed in kits for either 11 or 22 transformations. Reproducible high efficiencies are available in a variety of *E. coli* strains, including NovaBlue for routine cloning, blue/white screening and plasmid preparation, as well as pET expression strains (λDE3 lysogens), and isogenic control strains (non-lysogens) for superior performance in protein expression applications. The designation (DE3) indicates that the host is a lysogen of λDE3, and therefore carries a chromosomal copy of the T7 RNA polymerase gene under control of the *lacUV5* promoter (1–3). Such strains are suitable for production of protein from target genes cloned in pET vectors. pLysS is a designation given to hosts carrying a pET-compatible plasmid that encodes T7 lysozyme, which is a natural inhibitor of T7 RNA polymerase (4–5). This strain is used to suppress basal expression of T7 RNA polymerase prior to induction and thus stabilize pET recombinants encoding target proteins that affect cell growth and viability. The pLacI designation is given to hosts carrying a compatible plasmid that encodes *lac* repressor under control of its wild type promoter. Expression hosts carrying this plasmid were specifically designed for compatibility with the pETBlue™ and pTriEx™ plasmid series. The λDE3 Lysogenization Kit is also available for making new expression hosts with other genetic backgrounds.

### Components

#### Standard 0.4 ml and 1 ml Kits

- 2 × 0.2 ml or 5 × 0.2 ml competent cells
- 2 × 2 ml or 4 × 2 ml SOC Medium
- Test Plasmid (ampicillin resistant)

#### Singles Kits

- 11 × 50 µl or 22 × 50 µl competent cells
- 2 × 2 ml or 4 × 2 ml SOC Medium
- Test Plasmid (ampicillin resistant)

#### Storage

Store all components at –70°C or below.



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## Strain information

Strains offered as competent cells are listed in the table below. Genotypes are found on page 14.

Strains having the designation (DE3) are lysogenic for a λ prophage that contains an IPTG-inducible T7 RNA polymerase. λDE3 lysogens are designed for protein expression from pET, pETBlue™, and pTriEx™ vectors. Strains having the pLysS designation carry a pACYC184-derived plasmid that encodes T7 lysozyme, which is a natural inhibitor of T7 RNA polymerase that serves to repress basal expression of target genes under the control of the T7 promoter. Strains having the designation pLacI carry a pACYC-derived plasmid that encodes the *lac* repressor, which serves to suppress basal expression of target genes under T7 promoter control in pETBlue and pTriEx plasmids.

| Strain | Resistance[1] | Derivation | Key Feature(s) |
|---|---|---|---|
| AD494<br>AD494(DE3)<br>AD494(DE3)pLysS | Kan<br>Kan<br>Kan + Cam | K-12 | *trxB* mutant; facilitates cytoplasmic disulfide bond formation |
| BL21<br>BL21(DE3)<br>BL21(DE3)pLysS | none<br>none<br>Cam | B834 | *lon* and *ompT* protease deficient |
| BL21*trxB*(DE3)<br>BL21*trxB*(DE3)pLysS | Kan<br>Kan + Cam | BL21 | BL21 *trxB* mutant; facilitates cytoplasmic disulfide bond formation |
| BLR<br>BLR(DE3)<br>BLR(DE3)pLysS | Tet<br>Tet<br>Tet + Cam | BL21 | BL21 *recA* mutant; stabilizes tandem repeats |
| B834(DE3)<br>B834(DE3)pLysS | none<br>Cam | B strain | Met auxotroph; $^{35}$S-met labeling |
| HMS174<br>HMS174(DE3)<br>HMS174(DE3)pLysS | Rif<br>Rif<br>Rif + Cam | K-12 | *recA* mutant, Rif resistance |
| NovaBlue<br>NovaBlue(DE3) | Tet<br>Tet | K-12 | *recA*, *endA*, *lacI*$^q$ ; recommended for cloning, plasmid preps |
| Origami™<br>Origami(DE3)<br>Origami(DE3)pLysS<br>Origami(DE3)pLacI[2] | Kan + Tet<br>Kan + Tet<br>Kan + Tet + Cam<br>Kan + Tet + Cam | K-12 | *trxB*/*gor* mutant, greatly facilitates cytoplasmic disulfide bond formation |
| Origami B<br>Origami B(DE3)<br>Origami B(DE3)pLysS<br>Origami B(DE3)pLacI[2] | Kan + Tet<br>Kan + Tet<br>Kan + Tet + Cam<br>Kan + Tet + Cam | Tuner (B strain) | *trxB*/*gor* mutant, greatly facilitates cytoplasmic disulfide bond formation<br>BL21 *lacY* deletion mutant; allows “rheostat” control with IPTG |

(continued on next page)

| Strain | Resistance[1] | Derivation | Key Feature(s) |
|---|---|---|---|
| Rosetta™<br>Rosetta(DE3)<br>Rosetta(DE3)pLysS<br>Rosetta(DE3)pLacI[2] | Cam<br>Cam<br>Cam<br>Cam | Tuner<br>(B strain) | BL21 *lacY* deletion mutant; allows "rheostat" control with IPTG.<br>Expresses rare tRNAs; facilitates expression of genes that encode rare *E. coli* codons. |
| Rosetta-gami™<br>Rosetta-gami(DE3)<br>Rosetta-gami(DE3)pLysS<br>Rosetta-gami(DE3)pLacI[2] | Kan + Tet + Cam<br>Kan + Tet + Cam<br>Kan + Tet + Cam<br>Kan + Tet + Cam | Origami<br>(K-12) | Expresses rare tRNAs; facilitates expression of genes that encode rare *E. coli* codons<br>*trxB*/*gor* mutant, greatly facilitates cytoplasmic disulfide bond formation |
| RosettaBlue™<br>RosettaBlue(DE3)<br>RosettaBlue(DE3)pLysS<br>RosettaBlue(DE3)pLacI[2] | Tet + Cam<br>Tet + Cam<br>Tet + Cam<br>Tet + Cam | NovaBlue<br>(K-12) | Expresses rare tRNAs; facilitates expression of genes that encode rare *E. coli* codons<br>*recA*, *endA*, *lacI*$^q$ ; High transformation efficiency |
| Tuner™<br>Tuner(DE3)<br>Tuner(DE3)pLysS<br>Tuner(DE3)pLacI[2] | none<br>none<br>Cam<br>Cam | BL21 | BL21 *lacY* deletion mutant; allows "rheostat" control with IPTG |

1. The Resistance column in the table refers to selectable marker(s) possessed by the strain in the absence of target plasmids. Appropriate concentrations for selection are as follows:
   Kan = 15 µg/ml kanamycin
   Cam = 34 µg/ml chloramphenicol
   Tet = 12.5 µg/ml tetracycline
   Rif = 200 µg/ml rifampicin

2. Strains with the pLacI plasmid are appropriate hosts for pTriEx and pETBlue vectors only.

| Antibiotics/IPTG available separately | Size | Cat. No. |
|---|---|---|
| Carbenicillin | 5 g | 69101-3 |
| Chloramphenicol | 5 g | 220551 |
| Kanamycin | 25 g | 420311 |
| Tetracycline | 25 g | 58346 |
| 100 mM IPTG Solution | 10 × 1.5 ml | 70527-3 |

**AD494** strains provide the thioredoxin reductase (*trxB*) mutation in a K-12 background. Their *trxB* status allows disulfide bond formation to occur in the cytoplasm, providing the potential to produce properly folded, active proteins (6). The *trxB* mutation is selectable on kanamycin; therefore, this strain is not compatible with kanamycin resistant plasmids.

**B834** is the parental strain for BL21 (7). These hosts are methionine auxotrophs and allow high specific activity labeling of target proteins with $^{35}$S-methionine and selenomethionine for crystallography (8). This strain is also deficient in the *lon* (9) and *ompT* proteases.

**BL21** is the most widely used host background for protein expression and has the advantage of being deficient in both *lon* (9) and *ompT* proteases.



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**BL21*trxB*** strains possess the same thioredoxin reductase mutation (*trxB*) as the AD494 strains in the BL21 background. Since *trxB* hosts facilitate cytoplasmic disulfide bond formation, their use may increase the fraction of properly folded protein (10). The *trxB* mutation is selectable on kanamycin; therefore, these strains are not compatible with kanamycin resistant plasmids. These strains are also deficient in the *lon* and *ompT* proteases.

**BLR** is a *recA* derivative of BL21 (11) that improves plasmid monomer yields and may help stabilize target plasmids containing repetitive sequences or whose products may cause the loss of the DE3 prophage (12). These strains are also deficient in the *lon* and *ompT* proteases.

**HMS174** strains provide the *recA* mutation in a K-12 background. Like BLR, these strains may stabilize certain target genes whose products may cause the loss of the DE3 prophage.

**NovaBlue** is a K-12 strain ideally suited as an initial cloning host due to its high transformation efficiency, blue/white screening capability (with appropriate plasmids) and *recA endA* mutations, which result in high yields of excellent quality plasmid DNA. The DE3 lysogen of NovaBlue is potentially useful as a stringent host due to the presence of the *lacI*$^q$ repressor encoded by the F episome. Note, however that the DE3 lysogen cannot be used for blue/white screening of recombinant plasmids.

**Origami**™ host strains are K-12 derivatives that have mutations in both the thioredoxin reductase (*trxB*) and glutathione reductase (*gor*) genes, which greatly enhances disulfide bond formation in the cytoplasm (12, 13). Studies have shown that expression in Origami(DE3) yielded 10-fold more active protein than in a *trxB* single mutant host even though overall expression levels were similar. Origami hosts are not compatible with kanamycin or tetracycline resistant plasmids. Origami hosts are ideal for use with pET-32 vectors, since the thioredoxin fusion tag further enhances the formation of disulfide bonds in the cytoplasm (10). Origami strains do not support the replication of M13 phage or bacteriophage λCE6 based induction of expression.

**Origami™ B** host strains are derived from Tuner™ strains, which were in turn derived from BL21. Therefore, the Origami B hosts possess a unique combination of mutations that greatly facilitates the expression of intact, active, soluble proteins in *E. coli*. Mutations in the thioredoxin reductase (*trxB*) and glutathione reductase (*gor*) genes create a cytoplasmic environment compatible with the formation of disulfide bonds. Deletion of the *lac* permease gene (*lacY*) allows adjustable, inducer (IPTG) dependent and uniform expression of target protein throughout all cells in a culture. Deficiencies in the *lon* and *ompT* proteases help to reduce proteolytic degradation. Origami B hosts are not compatible with kanamycin or tetracycline resistant plasmids. Origami B hosts are ideal for use with pET-32 vectors, since the thioredoxin fusion tag further enhances the formation of disulfide bonds in the cytoplasm (10). Origami strains do not support the replication of M13 phage or bacteriophage λCE6 based induction of expression.

**Rosetta**™ host strains are designed to enhance the expression of eukaryotic proteins that contain codons rarely used in *E. coli* (14-17). Expression of these proteins can be dramatically increased when the level of rare tRNA is increased within the host (16-19). These strains supply tRNAs under the control of their native promoters for the codons AUA, AGG, AGA, CUA, CCC and GGA on a compatible chloramphenicol-resistant plasmid (17). Therefore this strain is not compatible with other chloramphenicol resistant plasmids. The Rosetta strains provide for "universal" translation which is otherwise limited by the codon usage of *E. coli* and alleviate the need to synthesize codon optimized genes. In the pLysS and pLacI Rosetta strains, the rare tRNA genes are present on the same plasmids that carry the T7 lysozyme and *lac* repressor genes, respectively. The Rosetta series is derived from the BL21 *lacY1* mutant Tuner strain.

**RosettaBlue™** has the NovaBlue background and a supply of tRNA's for codons rarely used in *E. coli* designed to enhance the expression of eukaryotic proteins. These strains supply tRNAs under the control of their native promoters for the codons AUA, AGG, AGA, CUA, CCC and GGA on a compatible chloramphenicol-resistant plasmid (17). This is a stringent host for protein expression due to the *lacI*$^q$ repressor mutation and has a high transformation efficiency. RosettaBlue strains are not compatible with tetracycline and chloramphenicol resistant plasmids. In the pLysS and pLacI RosettaBlue strains, the rare tRNA genes are present on the same plasmids that carry the T7 lysozyme and *lac* repressor genes, respectively.

**Rosetta-gami™** has the Origami™ background and a supply of tRNA's for codons rarely used in *E. coli* designed to enhance both the expression of eukaryotic proteins and the formation of

target protein disulfide bonds in the cytoplasm. These strains supply the tRNAs under the control of their native promoters for the codons AUA, AGG, AGA, CUA, CCC and GGA on a compatible chloramphenicol-resistant plasmid (17). The strain also has mutations in thioredoxin reductase (*trxB*) and glutathione reductase (*gor*) genes that greatly enhance disulfide bond formation in the cytoplasm (12, 13). Rosetta-gami hosts are ideal for use with pET-32 vectors, since the thioredoxin fusion tag further enhances the formation of disulfide bonds in the cytoplasm (10). These strains are not compatible with tetracycline, kanamycin or chloramphenicol resistant plasmids. In the pLysS and pLacI Rosetta-gami strains, the rare tRNA genes are present on the same plasmids that carry the T7 lysozyme and *lac* repressor genes, respectively. Origami strains do not support the replication of M13 phage or bacteriophage λCE6 based induction of expression.

**Tuner** strains are *lacY* deletion mutants of BL21 which enable adjustable levels of protein expression throughout all cells in a culture. The *lac* permease (*lacY*) mutation allows uniform entry of IPTG into all cells in the population. Unlike lactose (or arabinose), IPTG is a gratuitous inducer that can enter *E. coli* cells independently of permease pathways. This allows induction with IPTG to occur in a true concentration-dependent fashion that is exceptionally uniform throughout the culture. By adjusting the concentration of IPTG, expression can be regulated from very low expression levels up to the robust fully induced expression levels commonly associated with pET vectors. Lower level expression may enhance the solubility and activity of difficult target proteins. These strains are also deficient in the *lon* and *ompT* proteases.

## Transformation Protocol for Experienced Users

*Note:* *See the following section for a detailed protocol.*

1. Thaw the required number of tubes of cells on ice and mix gently to ensure that the cells are evenly suspended.
2. *Standard Kits:*
   Place the required number of 1.5-ml polypropylene microcentrifuge tubes on ice to pre-chill. Pipet 20 µl aliquots of cells into the pre-chilled tubes.

   *Singles™ Kits:*
   Proceed to Step 3.
3. Add 1 µl of the DNA solution directly to the cells. Stir gently to mix.
4. Place the tubes on ice for 5 min.
5. Heat the tubes for exactly 30 sec in a 42°C water bath; do not shake.
6. Place on ice for 2 min.
7. *Standard Kits:*
   Add **80 µl** of room temperature SOC Medium to each tube.

   *Singles Kits:*
   Add **250 µl** of room temperature SOC Medium to each tube.
8. Selection for transformants is accomplished by plating on media containing antibiotic for the plasmid encoded drug resistance. Additional host-specific antibiotics may also be appropriate to insure maintenance of the host encoded feature(s) (see chart on page 3).

   **When using NovaBlue:** if selecting for ampicillin resistance, plate 5–50 µl cells directly on selective medium (plus IPTG/X-gal for plasmids that permit blue/white screening). If selecting for kanamycin resistance, shake at 37°C (250 rpm) for 30 min prior to plating on selective medium.

   **When using strains other than NovaBlue:** incubate at 37°C while shaking at 250 rpm for 60 min prior to plating on selective medium.



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## Transformation - Detailed Protocol

*Note:* *When selecting for the expression of β-lactamase, the antibiotic carbenicillin is recommended over ampicillin. Carbenicillin is less sensitive to the drop in the pH of the growth medium that typically accompanies bacterial growth.*

Competent cells in the standard kits are provided in 0.2 ml aliquots. The standard transformation reaction calls for 20 µl cells, so each tube contains enough cells for 10 transformations. Singles™ competent cells are provided in 50 µl aliquots, which are used "as is" for single 50 µl transformations. Please note that there are a few steps in the protocol that vary for the Singles vs. standard cells.

DNA in ligation reactions containing high-quality reagents is suitable for direct addition to Novagen's competent cells. Inactivation of the ligase is not required prior to transformation. Plasmid DNA isolated using standard miniprep procedures is also usually satisfactory; however, for maximum efficiency, the sample DNA should be free of phenol, ethanol, salts, protein and detergents, and dissolved in TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA) or in water. No more than 1 µl of ligation reaction should be used per 20 µl of competent cells to insure optimal transformation efficiency.

### Handling tips

1. Upon receipt from Novagen, verify that the competent cells are still frozen and that dry ice is still present in the shipping container. Immediately place the competent cells at –70°C or below. For optimal results, do not allow the cells to thaw at any time prior to use.
2. Handle only the very top of the tube and the tube cap to prevent the cells from warming. Keep the cells on ice whenever possible.
3. To mix cells, flick the tube 1–3 times. *NEVER vortex the competent cells*.
4. Cells can be refrozen at –70°C and reused; however, transformation efficiencies may decline several-fold with each freeze-thaw cycle. To avoid multiple freeze-thaw cycles of the standard cells, dispense the cells into aliquots after the initial thaw and store them at –70°C or below (note that Singles cells are provided as 50 µl aliquots, which are used "as is" and should not be sub-divided). To dispense aliquots of cells from the 0.2 ml stock, remove the stock tube quickly from the ice and flick 1–2 times to mix prior to opening the tube. Remove a 20 µl aliquot from the middle of the cells, and replace the tube immediately on ice. Place the aliquot immediately into the bottom of a pre-chilled 1.5 ml tube, mix by pipetting <u>once</u> up and down, and then immediately close the tube and replace on ice. After all of the aliquots are taken, return any unused tubes to the freezer before proceeding with the transformation.

### Procedure

5. Remove the appropriate number of competent cell tubes from the freezer (include one extra sample for the Test Plasmid positive control, if desired). Immediately place the tubes on ice, so that all but the cap is surrounded by ice. Allow the cells to thaw on ice for ~2–5 minutes.
6. Visually check the cells to see that they have thawed and gently flick the cells 1–2 times to evenly resuspend the cells. The cells are then ready for removal of an aliquot (Standard Kits), or for the addition of the DNA (*Singles*™ Kits).

| | |
|---|---|
| 3. <u>*Standard Kits:*</u><br>Place the required number of 1.5-ml snap-cap polypropylene tubes on ice to pre-chill. Pipet 20 µl aliquots of cells into the pre-chilled tubes. | <u>*Singles Kits:*</u><br>Proceed to Step 4 or 5, depending on whether a Test Plasmid sample is included as a positive control. |

4. (Optional) To determine transformation efficiency, add 1 µl (0.2 ng) Test Plasmid to one of the tubes containing cells. Stir gently to mix and return the tube to the ice.
5. Add 1 µl of a ligation reaction or purified plasmid DNA directly to the cells. Stir gently to mix and return the tube to the ice, making sure that the tube is surrounded by ice except for the cap. Repeat for additional samples.

*Note:* *Transformation efficiencies can be increased several fold by diluting the ligation reaction 5-fold with TE or water prior to adding the DNA, or by extracting the ligation reaction twice with 1:1 TE-buffered phenol:CIAA (24:1 chloroform:isoamyl alcohol), once with CIAA, precipitating in the presence of NaOAc, and resuspending in TE or water before adding the DNA to the cells.*

6. Incubate the tubes on ice for 5 min.
7. Heat the tubes for exactly 30 sec in a 42°C water bath; do not shake.

*Note:* *This "heat shock" step is most easily accomplished if the tubes are in a rack that leaves the lower half of the tubes exposed. Hold the rack in the water bath so that the lower halves of the tubes are submerged for 30 sec, and then replace the rack on ice.*

8. Place the tubes on ice for 2 min.
9. | *Standard Kits:* | *Singles™ Kits:* |
   |---|---|
   | Add **80 µl** of room temperature SOC medium to each tube. Keep the tubes on ice until all have received SOC. | Add **250 µl** of room temperature SOC medium to each tube. Keep the tubes on ice until all have received SOC. |
10. Selection for transformants is accomplished by plating on medium containing antibiotic for the plasmid encoded drug resistance. Additional host-specific antibiotics may also be appropriate to insure maintenance of the host encoded feature(s) (see chart on page 3).

    **When using NovaBlue:** if selecting for ampicillin resistance, plate 5–50 µl cells directly on selective media (plus IPTG/X-gal for plasmids which permit blue/white screening). If selecting for kanamycin resistance, shake at 37°C (250 rpm) for 30 min prior to plating on selective media.
    **When using strains other than NovaBlue:** incubate at 37°C while shaking at 250 rpm for 60 min prior to plating on selective media.

*Notes:* *The outgrowth incubation is conveniently performed in a shaking incubator using a test tube rack anchored to the shaking platform. Place each transformation tube in an empty 13 mm × 100 mm glass test tube in the rack. The snap-caps on the transformation tubes prevent them from falling to the bottom of the test tubes, and all transformation tubes remain vertical.*

*During the outgrowth (or earlier if omitting outgrowth), place the plates at 37°C. If the plates contain a lot of moisture, place them cover-side up and open the cover ~1/3 of the way to allow the plates to dry for 30–45 min. If the plates do not need drying, keep them closed and place them cover-side down in the 37°C incubator for ~20 min prior to plating.*

11. Refer to "Plating Technique" in the subsequent section for specific instructions. Spread 5–50 µl of each transformation on LB agar plates containing the appropriate antibiotic. Use 50 µg/ml carbenicillin or ampicillin for amp-resistant vectors (which encode β-lactamase), or 30 µg/ml kanamycin for kan-resistant vectors (which encode aminoglucoside 3′-phosphotransferase). The plates should also contain 34 µg/ml chloramphenicol if a strain carrying pLysS or pLacI is used. When plating less than 25 µl, first pipet a "pool" of SOC onto the plate and then pipet the cells into the SOC. Please see the next section for additional details on plating technique.

*Important:* *The appropriate amount of transformation mixture to plate varies with the efficiency of both the ligation and the competent cells. As little as 2 µl will yield several hundred transformants under highly efficient conditions (e.g., with NovaBlue cells giving > 4 × $10^8$ cfu/µg). For recombinants in NovaBlue, expect $10^5$–$10^7$ transformants/µg plasmid, depending on the particular insert and the ligation efficiency.*

***When using the Test Plasmid**, plate no more than 5 µl of the final NovaBlue transformation mix (e.g., 5 µl of NovaBlue cells at 1 × $10^8$ efficiency) or plate 10 µl of any strain with a 2 × $10^6$ efficiency in a pool of SOC on an LB agar plate containing 50 µg/ml carbenicillin or ampicillin (because the Test Plasmid carries the ampicillin resistance gene, bla).*

***For blue/white screening of recombinants**, also include IPTG and X-gal in the LB agar. These can be pre-spread on the plates and allowed to soak in for about 30 min prior to plating. Use 35 µl of 50 mg/ml X-gal in dimethyl formamide and 20 µl 100 mM IPTG (in water) per 82 mm plate. Alternatively, X-gal and IPTG can be added to the LB agar at a final concentration of 70 µg/ml and 80 µM, respectively, just prior to pouring the plates.*

12. Let the plates sit on the bench for several min to allow excess liquid to be absorbed, and then invert and incubate overnight at 37°C.



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## Plating techniques

1. Remove the plates from the incubator. If plating less than 25 µl of the transformation, we recommend plating onto a pool of SOC, which facilitates even colony distribution on the plate surface. Using a sterile pipet tip, place 40–60 µl of SOC in the center of a plate for a plating cushion.
2. To remove the transformation sample, flick the transformation tube 5–8 times, open the cap and immediately remove the sample volume from the middle of the transformation reaction.
3. Transfer the sample to the plate by dispensing the sample volume into the SOC cushion. After the sample is out of the pipet tip, use the same tip to pipet up the sample volume's worth of SOC from the cushion edge and dispense that SOC back into the cushion. (This effectively rinses out your pipet tip.)

*ColiRollers Plating Beads are glass beads that eliminate the use of the spreader and alcohol flame while evenly distributing cells without the possibility of damage.*

### Plating with ColiRollers™ Plating Beads

To use ColiRollers, simply dispense 10–20 beads per plate. Cover the plate with its lid and move the plate back and forth several times. The rolling action of the beads distributes the cells. Several plates can be stacked up and shaken at one time. After all plates have been spread, discard the ColiRollers and incubate (step 7 below).

### Plating with a standard spreader

4. Completely immerse the plating spreader (bent glass rod or equivalent) into ethanol and flame to sterilize. After the flame is extinguished, allow the spreader to cool ~10 seconds prior to placing the spreader on the plate. Place the spreader on the LB agar at the outside of the plate (not touching the pool of cells). This further cools the spreader on the LB agar before spreading the cells.
5. *Slowly* turn the plate while supporting the weight of the spreader.

*Important: Do not press down on the spreader – use just enough pressure to spread the cells.*

6. Spread until the sample is evenly distributed on the plate. If the plates are fairly dry, the sample and cushion will quickly absorb into the plate. Once the moisture is absorbed, do not continue spreading. If the plates are wet, spread until the sample is evenly distributed. Do not continue to spread until the sample and cushion have absorbed completely into the plate, as overspreading is lethal to the cells. Instead, after spreading briefly, allow the plates to sit upright at room temperature for ~15 minutes prior to placing them in the 37ºC incubator. This will allow excess moisture to absorb into the plates before the plates are inverted and placed in the incubator.
7. Incubate all plates, cover-side down, in the 37ºC incubator for 15–18 hours. To obtain larger colonies, extend the incubation time slightly (1–2 hours), but beware of the potential for development of satellite colonies with extended incubations (usually > 36 h at 37°C; satellites are not commonly observed when using carbenicillin or kanamycin). If performing blue/white screening, placing the plates in a 4ºC refrigerator for a few hours after the colonies have reached the desired size can enhance blue color development.

## Troubleshooting

Problems rarely occur if the above protocols are carefully followed. The Test Plasmid is included with all Novagen competent cells to use as a positive control, and provides useful information if problems are encountered.

| Problem | Possible solution |
|---|---|
| Experimental DNA produces no colonies or very low number of colonies, but Test Plasmid (included with the kit) yields expected efficiency | a. Experimental DNA contains an inhibitor of ligation. Make sure input DNA is free of contaminants (e.g. excess salts, EDTA, proteins, etc.) that inhibit ligation. Gel purify and/or extract the vector and insert prior to ligation.<br>b. Experimental DNA contains an inhibitor of transformation. Mix Test Plasmid with the ligation and transform on ampicillin plates. If the expected number of colonies is seen, this is not the problem. If colony number is low, dilute the ligation 5-fold in TE buffer or extract, precipitate and resuspend the ligation in TE buffer prior to transformation. Note that only $amp^R$ plasmid can be mixed with the $amp^R$ test plasmid.<br>c. Vector and/or insert have damaged or otherwise incompatible ends. Recheck cloning strategy, including vector:insert ratio, and use fresh, reliable reagents for DNA preparation. If cloning PCR products, it is likely to be faster to clone them first using a Perfectly Blunt® or **A**ccep**T**or™ Vector Kit. Then, if needed, transfer into another vector using restriction enzymes to excise the fragment.<br>d. Insert is not tolerated in *E. coli*. If possible, check the target sequence for strong *E. coli* promoters or other potentially toxic elements, as well as inverted repeats. Occasionally, certain repeated elements (usually found only in genomic DNA) are not well-maintained in NovaBlue or other multi-purpose *E. coli* strains. These sequences can sometimes be cloned in *recJ*$^-$ strains.<br>e. Verify that IPTG was NOT added to the plate when attempting to transform a DE3 lysogen based host strain. IPTG will induce the expression of T7 RNA polymerase in DE3 hosts and any target gene on a T7 promoter based plasmid. This typically results in decreased fitness of the cell and will likely be selected against over time. IPTG induction of DE3 hosts should ONLY be performed after a stable transformant has been isolated.<br>f. Avoid exceeding the recommended volume of input DNA. DNA volumes greater than 1 µl per 20 µl of competent cells will lead to reduced transformation efficiencies. |
| No colonies or low colony numbers with the Test Plasmid | a. If no colonies are observed, the incorrect selective drug or the wrong concentration of the correct selective drug may have been used in the plates. Use ampicillin or carbenicillin at 50 µg/ml with the Test Plasmid.<br>b. Incorrect or toxic media components, or plates too old/dry. Recheck media formulations.<br>c. Incorrect incubator temperature. Make sure incubator is set to 37°C.<br>d. Cells were handled incorrectly. Handle the cells very gently at all times. Never vortex or mix vigorously. To resuspend cells, finger-flick or gently pipet up and down without generating bubbles. Make sure the cells are stored at –70°C or below. Thaw on ice and keep on ice except where indicated in the procedure. Gently resuspend the cells before plating if they settle out during outgrowth. Use a very light touch with the spreader when plating or use ColiRollers™ Plating Beads. |
| Small “satellite” colonies present | a. Plates were left at 37°C too long. β-lactamase is secreted by amp-resistant bacteria and thus can eventually clear a zone of surrounding media from the drug, allowing non-recombinants to grow. In general, colonies are sufficiently large for analysis after 18 h (NovaBlue) or 15 h (other strains).<br>b. Ampicillin stock is degraded, plates are old or drug was added when the media was too hot. Use freshly prepared ampicillin and correct plate preparation. Alternatively, use the ampicillin analog carbenicillin, which appears to be less susceptible to degradation. |



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## Ordering Information

| pET Expression Strains: λDE3 Lysogens | Size | Cat. No. |
|---|---|---|
| AD494(DE3) Competent Cells | 0.4 ml | 69013-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 69013-4 |
| AD494(DE3)pLysS Competent Cells | 0.4 ml | 69014-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 69014-4 |
| B834(DE3) Competent Cells | 0.4 ml | 69041-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 69041-4 |
| B834(DE3)pLysS Competent Cells | 0.4 ml | 69042-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 69042-4 |
| BL21(DE3) Competent Cells | 0.4 ml | 69450-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 69450-4 |
| BL21(DE3)pLysS Competent Cells | 0.4 ml | 69451-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 69451-4 |
| BL21*trxB*(DE3) Competent Cells | 0.4 ml | 70508-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 70508-4 |
| BL21*trxB*(DE3)pLysS Competent Cells | 0.4 ml | 70509-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 70509-4 |
| BLR(DE3) Competent Cells | 0.4 ml | 69053-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 69053-4 |
| BLR(DE3)pLysS Competent Cells | 0.4 ml | 69956-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 69956-4 |
| HMS174(DE3) Competent Cells | 0.4 ml | 69453-3 |
| guaranteed transformation efficiency $5 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 69453-4 |
| HMS174(DE3)pLysS Competent Cells | 0.4 ml | 69454-3 |
| guaranteed transformation efficiency $5 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 69454-4 |
| NovaBlue(DE3) Competent Cells | 0.4 ml | 69284-3 |
| guaranteed transformation efficiency $1 \times 10^8$ cfu/µg Test Plasmid | 1 ml | 69284-4 |
| Origami™(DE3) Competent Cells | 0.4 ml | 70627-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 70627-4 |
| Origami(DE3)pLysS Competent Cells | 0.4 ml | 70628-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 70628-4 |
| Origami(DE3)pLacI Competent Cells | 0.4 ml | 70629-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 70629-4 |
| Origami™ B(DE3) Competent Cells | 0.4 ml | 70837-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 70837-4 |
| Origami B(DE3)pLysS Competent Cells | 0.4 ml | 70839-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 70839-4 |
| Origami B(DE3)pLacI Competent Cells | 0.4 ml | 70838-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 70838-4 |
| Rosetta™(DE3) Competent Cells | 0.4 ml | 70954-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 70954-4 |
| Rosetta(DE3)pLysS Competent Cells | 0.4 ml | 70956-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 70956-4 |
| Rosetta(DE3)pLacI Competent Cells | 0.4 ml | 70920-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 70920-4 |
| Rosetta-gami™(DE3) Competent Cells | 0.4 ml | 71055-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 71055-4 |

| pET Expression Strains: λDE3 Lysogens (continued) | Size | Cat. No. |
|---|---|---|
| Rosetta-gami™(DE3)pLysS Competent Cells<br>guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 0.4 ml<br>1 ml | 71057-3<br>71057-4 |
| Rosetta-gami(DE3)pLacI Competent Cells<br>guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 0.4 ml<br>1 ml | 71056-3<br>71056-4 |
| RosettaBlue™(DE3) Competent Cells<br>guaranteed transformation efficiency $1 \times 10^8$ cfu/µg Test Plasmid | 0.4 ml<br>1 ml | 71059-3<br>71059-4 |
| RosettaBlue(DE3)pLysS Competent Cells<br>guaranteed transformation efficiency $1 \times 10^8$ cfu/µg Test Plasmid | 0.4 ml<br>1 ml | 71034-3<br>71034-4 |
| RosettaBlue(DE3)pLacI Competent Cells<br>guaranteed transformation efficiency $1 \times 10^8$ cfu/µg Test Plasmid | 0.4 ml<br>1 ml | 71060-3<br>71060-4 |
| Tuner™(DE3) Competent Cells<br>guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 0.4 ml<br>1 ml | 70623-3<br>70623-4 |
| Tuner(DE3)pLysS Competent Cells<br>guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 0.4 ml<br>1 ml | 70624-3<br>70624-4 |
| Tuner(DE3)pLacI Competent Cells<br>guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 0.4 ml<br>1 ml | 70625-3<br>70625-4 |

| pET Competent Cell Sets | Cat. No. |
|---|---|
| (DE3) Competent Cell Set<br>AD494(DE3), BL21(DE3), BL21*trxB*(DE3), BLR(DE3), HMS174(DE3), NovaBlue(DE3), Origami™(DE3), Origami B(DE3), Rosetta™(DE3), Tuner™(DE3): 0.2 ml each, SOC & Test Plasmid | 71032-3 |
| (DE3)pLysS Competent Cell Set<br>AD494(DE3)pLysS, BL21(DE3)pLysS, BL21*trxB*(DE3)pLysS, BLR(DE3)pLysS, HMS174(DE3)pLysS, Origami(DE3)pLysS, Origami B(DE3)pLysS, Rosetta(DE3)pLysS, Tuner(DE3)pLysS: 0.2 ml each SOC & Test Plasmid | 71033-3 |
| AD494 Competent Cell Set<br>AD494, AD494(DE3), AD494(DE3)pLysS: 2 × 0.2 ml each, SOC & Test Plasmid | 70231-3 |
| BL21 Competent Cell Set<br>BL21, BL21(DE3), BL21(DE3)pLysS: 2 × 0.2 ml each, SOC & Test Plasmid | 70232-3 |
| BLR Competent Cell Set<br>BLR, BLR(DE3), BLR(DE3)pLysS: 2 × 0.2 ml each, SOC & Test Plasmid. | 70233-3 |
| HMS174 Competent Cell Set<br>HMS174, HMS174(DE3), HMS174(DE3)pLysS: 2 × 0.2 ml each, SOC & Test Plasmid | 70234-3 |
| Origami Competent Cell Set<br>Origami, Origami(DE3), Origami(DE3)pLysS: 2 × 0.2 ml each, SOC & Test Plasmid | 70670-3 |
| Origami B Competent Cell Set<br>Origami B, Origami B(DE3), Origami B(DE3)pLysS: 2 × 0.2 ml each, SOC & Test Plasmid | 70911-3 |
| Rosetta Competent Cell Set<br>Rosetta, Rosetta(DE3), Rosetta(DE3)pLysS: 2 × 0.2 ml each, SOC & Test Plasmid | 70987-3 |
| **pET Competent Cell Sets (continued)** | **Cat. No.** |



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| | |
|---|---|
| RosettaBlue Competent Cell Set<br>RosettaBlue, RosettaBlue(DE3), RosettaBlue(DE3)pLysS 2 × 0.2 ml each, SOC & Test Plasmid | 71079-3 |
| Rosetta-gami Competent Cell Set<br>Rosetta-gami, Rosetta-gami(DE3), Rosetta-gami(DE3)pLysS 2 × 0.2 ml each, SOC & Test Plasmid | 71080-3 |
| Tuner Competent Cell Set<br>Tuner, Tuner(DE3), Tuner(DE3)pLysS: 2 × 0.2 ml each, SOC & Test Plasmid | 70726-3 |

| pETBlue™ and pTriEx™ Expression Strains | Size | Cat. No. |
|---|---|---|
| Origami™(DE3)pLacI Competent Cells | 0.4 ml | 70629-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 70629-4 |
| Origami B(DE3)pLacI Competent Cells | 0.4 ml | 70838-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 70838-4 |
| Rosetta™(DE3)pLacI Competent Cells | 0.4 ml | 70920-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 70920-4 |
| Rosetta-gami™(DE3)pLacI Competent Cells | 0.4 ml | 71056-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 71056-4 |
| RosettaBlue™(DE3)pLacI Competent Cells | 0.4 ml | 71060-3 |
| guaranteed transformation efficiency $1 \times 10^8$ cfu/µg Test Plasmid | 1 ml | 71060-4 |
| Tuner™(DE3)pLacI Competent Cells | 0.4 ml | 70625-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 70625-4 |

| Isogenic Strains (non-λDE3 Lysogens) | Size | Cat. No. |
|---|---|---|
| AD494 Competent Cells | 0.4 ml | 69033-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 69033-4 |
| BL21 Competent Cells | 0.4 ml | 69449-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 69449-4 |
| BLR Competent Cells | 0.4 ml | 69052-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 69052-4 |
| HMS174 Competent Cells | 0.4 ml | 69452-3 |
| guaranteed transformation efficiency $5 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 69452-4 |
| NovaBlue Competent Cells | 0.4 ml | 69825-3 |
| guaranteed transformation efficiency $1 \times 10^8$ cfu/µg Test Plasmid | 1 ml | 69825-4 |
| Origami Competent Cells | 0.4 ml | 70626-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 70626-4 |
| Origami B Competent Cells | 0.4 ml | 70836-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 70836-4 |
| Rosetta Competent Cells | 0.4 ml | 70953-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 70953-4 |
| Rosetta-gami Competent Cells | 0.4 ml | 71054-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 71054-4 |
| RosettaBlue Competent Cells | 0.4 ml | 71058-3 |
| guaranteed transformation efficiency $1 \times 10^8$ cfu/µg Test Plasmid | 1 ml | 71058-4 |
| Tuner Competent Cells | 0.4 ml | 70622-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 1 ml | 70622-4 |

| Singles™ Competent Cells | Size | Cat. No. |
|---|---|---|
| BL21(DE3) Singles Competent Cells | 11 rxn | 70235-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 22 rxn | 70235-4 |
| BL21(DE3)pLysS Singles Competent Cells | 11 rxn | 70236-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 22 rxn | 70236-4 |
| NovaBlue Singles Competent Cells | 11 rxn | 70181-3 |
| guaranteed transformation efficiency $1.5 \times 10^8$ cfu/µg Test Plasmid | 22 rxn | 70181-4 |
| Origami™(DE3) Singles Competent Cells | 11 rxn | 70630-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 22 rxn | 70630-4 |
| Origami(DE3)pLysS Singles Competent Cells | 11 rxn | 70631-3 |
| guaranteed transformation efficiency $2 \times 10^6$ cfu/µg Test Plasmid | 22 rxn | 70631-4 |

## Strain Genotypes

| Strain | Genotype |
|---|---|
| AD494 | Δ*ara–leu7697* Δ*lacX74* Δ*phoAPvuII phoR* Δ*malF3* F'[$lac^+(lacI^q)pro$] *trxB*::kan |
| AD494(DE3) | Δ*ara–leu7697* Δ*lacX74* Δ*phoAPvuII phoR* Δ*malF3* F'[$lac^+(lacI^q)pro$] *trxB*::kan (DE3) |
| AD494(DE3)pLysS | Δ*ara–leu7697* Δ*lacX74* Δ*phoAPvuII phoR* Δ*malF3* F'[$lac^+(lacI^q)pro$] *trxB*::kan (DE3) pLysS ($Cm^R$) |
| B834 | $F^-$ *ompT* $hsdS_B(r_B^- m_B^-)$ *gal dcm met* |
| B834(DE3) | $F^-$ *ompT* $hsdS_B(r_B^- m_B^-)$ *gal dcm met* (DE3) |
| B834(DE3)pLysS | $F^-$ *ompT* $hsdS_B(r_B^- m_B^-)$ *gal dcm met* (DE3) pLysS ($Cm^R$) |
| BL21 | $F^-$ *ompT* $hsdS_B(r_B^- m_B^-)$ *gal dcm* |
| BL21(DE3) | $F^-$ *ompT* $hsdS_B(r_B^- m_B^-)$ *gal dcm* (DE3) |
| BL21(DE3)pLysS | $F^-$ *ompT* $hsdS_B(r_B^- m_B^-)$ *gal dcm* (DE3) pLysS ($Cm^R$) |
| BL21(DE3)pLacI | $F^-$ *ompT* $hsdS_B(r_B^- m_B^-)$ *gal dcm* (DE3) pLacI ($Cm^R$) |
| BL21*trxB*(DE3) | $F^-$ *ompT* $hsdS_B(r_B^- m_B^-)$ *gal dcm trxB15*::kan (DE3) |
| BL21*trxB*(DE3)pLysS | $F^-$ *ompT* $hsdS_B(r_B^- m_B^-)$ *gal dcm trxB15*::kan (DE3) pLysS ($Cm^R$) |
| BLR | $F^-$ *ompT* $hsdS_B(r_B^- m_B^-)$ *gal dcm* Δ(*srl-recA*)*306*::Tn*10* ($Tc^R$) |
| BLR(DE3) | $F^-$ *ompT* $hsdS_B(r_B^- m_B^-)$ *gal dcm* Δ(*srl-recA*)*306*::Tn*10* ($Tc^R$) (DE3) |
| BLR(DE3)pLysS | $F^-$ *ompT* $hsdS_B(r_B^- m_B^-)$ *gal dcm* Δ(*srl-recA*)*306*::Tn*10* ($Tc^R$) (DE3) pLysS ($Cm^R$) |
| HMS174 | $F^-$ *recA1 hsdR*($r_{K12}^- m_{K12}^+$) $Rif^R$ |
| HMS174(DE3) | $F^-$ *recA1 hsdR*($r_{K12}^- m_{K12}^+$) $Rif^R$ (DE3) |
| HMS174(DE3)pLysS | $F^-$ *recA1 hsdR*($r_{K12}^- m_{K12}^+$) $Rif^R$ (DE3) pLysS ($Cm^R$) |
| NovaBlue | *endA1 hsdR17*($r_{K12}^- m_{K12}^+$) *supE44 thi-1 recA1 gyrA96 relA1 lac* F'[$proA^+B^+$ $lacI^qZ\Delta M15$::Tn*10* ($Tc^R$)] |
| NovaBlue(DE3) | *endA1 hsdR17*($r_{K12}^- m_{K12}^+$) *supE44 thi-1 recA1 gyrA96 relA1 lac* F'[$proA^+B^+$ $lacI^qZ\Delta M15$::Tn*10* ($Tc^R$)] (DE3) |
| Origami[1] | Δ*ara–leu7697 araD139* Δ*lacX74 galE galK rpsL* Δ*phoAPvuII phoR* F'[$lac^+(lacI^q)pro$] *gor522*::Tn*10* ($Tc^R$) *trxB*::kan |
| Origami(DE3)[1] | Δ*ara–leu7697 araD139* Δ*lacX74 galE galK rpsL* Δ*phoAPvuII phoR* F'[$lac^+(lacI^q)pro$] *gor522*::Tn*10* ($Tc^R$) *trxB*::kan (DE3) |
| Origami(DE3)pLysS[1] | Δ*ara–leu7697 araD139* Δ*lacX74 galE galK rpsL* Δ*phoAPvuII phoR* F'[$lac^+(lacI^q)pro$] *gor522*::Tn*10* ($Tc^R$) *trxB*::kan (DE3) pLysS ($Cm^R$) |
| Origami(DE3)pLacI[1] | Δ*ara–leu7697 araD139* Δ*lacX74 galE galK rpsL* Δ*phoAPvuII phoR* F'[$lac^+(lacI^q)pro$] *gor522*::Tn*10* ($Tc^R$) *trxB*::kan (DE3) pLacI ($Cm^R$) |



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| Strain | Genotype |
|---|---|
| Origami™ B | F⁻ *ompT hsdS*$_B$($r_B^-$ $m_B^-$) *gal dcm lacY1 gor522*::Tn*10* ($Tc^R$) *trxB*::kan |
| Origami B(DE3) | F⁻ *ompT hsdS*$_B$($r_B^-$ $m_B^-$) *gal dcm lacY1* (DE3) *gor522*::Tn*10* ($Tc^R$) *trxB*::kan (DE3) |
| Origami B(DE3)pLysS | F⁻ *ompT hsdS*$_B$($r_B^-$ $m_B^-$) *gal dcm lacY1* (DE3) pLysS ($Cm^R$) *gor522*::Tn*10* ($Tc^R$) *trxB*::kan (DE3) pLysS ($Cm^R$) |
| Origami B(DE3)pLacI | F⁻ *ompT hsdS*$_B$($r_B^-$ $m_B^-$) *gal dcm lacY1* (DE3) pLacI ($Cm^R$) *gor522*::Tn*10* ($Tc^R$) *trxB*::kan (DE3) pLacI ($Cm^R$) |
| Rosetta™ | F⁻ *ompT hsdS*$_B$($r_B^-$ $m_B^-$) *gal dcm lacY1* pRARE[2] (*(*$Cm^R$) |
| Rosetta(DE3) | F⁻ *ompT hsdS*$_B$($r_B^-$ $m_B^-$) *gal dcm lacY1* (DE3) pRARE[2] (*(*$Cm^R$) |
| Rosetta(DE3)pLysS | F⁻ *ompT hsdS*$_B$($r_B^-$ $m_B^-$) *gal dcm lacY1* (DE3) pLysSRARE[2] ($Cm^R$) |
| Rosetta(DE3)pLacI | F⁻ *ompT hsdS*$_B$($r_B^-$ $m_B^-$) *gal dcm lacY1* (DE3) pLacIRARE[2] ($Cm^R$) |
| Rosetta-gami™[1] | Δ*ara–leu7697* Δ*lacX74* Δ*phoAPvuII phoR araD139 galE galK rpsL* F'[*lac*$^+$(*lacI*$^q$)*pro*] *gor522* ::Tn*10* ($Tc^R$) *trxB*::kan pRARE[2] ($Cm^R$) |
| Rosetta-gami(DE3)[1] | Δ*ara–leu7697* Δ*lacX74* Δ*phoAPvuII phoR araD139 galE galK rpsL* F'[*lac*$^+$(*lacI*$^q$)*pro*] *gor522* ::Tn*10* ($Tc^R$) *trxB*::kan (DE3) pRARE[2] ($Cm^R$) |
| Rosetta-gami(DE3)pLysS[1] | Δ*ara–leu7697* Δ*lacX74* Δ*phoAPvuII phoR araD139 galE galK rpsL* F'[*lac*$^+$(*lacI*$^q$)*pro*] *gor522* ::Tn*10* ($Tc^R$) *trxB*::kan (DE3) pLysSRARE[2] ($Cm^R$) |
| Rosetta-gami(DE3)pLacI[1] | Δ*ara–leu7697* Δ*lacX74* Δ*phoAPvuII phoR araD139 galE galK rpsL* F'[*lac*$^+$(*lacI*$^q$)*pro*] *gor522* ::Tn*10* ($Tc^R$) *trxB*::kan (DE3) pLacIRARE[2] ($Cm^R$) |
| RosettaBlue™ | *endA1 hsdR17*($r_{K12}^-$ $m_{K12}^+$) *supE44 thi-1 recA1 gyrA96 relA1 lac* pRARE[2] ($Cm^R$) [F' *proA*$^+$*B*$^+$ *lacI*$^q$*ZΔM15* ::Tn*10*($Tc^R$)] |
| RosettaBlue(DE3) | *endA1 hsdR17*($r_{K12}^-$ $m_{K12}^+$) *supE44 thi-1 recA1 gyrA96 relA1 lac* pRARE[2] ($Cm^R$) [F' *proA*$^+$*B*$^+$ *lacI*$^q$*ZΔM15* ::Tn*10*($Tc^R$)] (DE3) |
| RosettaBlue(DE3)pLysS | *endA1 hsdR17*($r_{K12}^-$ $m_{K12}^+$) *supE44 thi-1 recA1 gyrA96 relA1 lac* pLysSRARE[2] ($Cm^R$)[F' *proA*$^+$*B*$^+$ *lacI*$^q$*ZΔM15* ::Tn*10*($Tc^R$)] (DE3) |
| RosettaBlue(DE3)pLacI | *endA1 hsdR17*($r_{K12}^-$ $m_{K12}^+$) *supE44 thi-1 recA1 gyrA96 relA1 lac* pLacIRARE[2] ($Cm^R$)[F' *proA*$^+$*B*$^+$ *lacI*$^q$*ZΔM15* ::Tn*10*($Tc^R$)] (DE3) |
| Tuner™ | F⁻ *ompT hsdS*$_B$($r_B^-$ $m_B^-$) *gal dcm lacY1* |
| Tuner(DE3) | F⁻ *ompT hsdS*$_B$($r_B^-$ $m_B^-$) *gal dcm lacY1* (DE3) |
| Tuner(DE3)pLysS | F⁻ *ompT hsdS*$_B$($r_B^-$ $m_B^-$) *gal dcm lacY1* (DE3) pLysS ($Cm^R$) |
| Tuner(DE3)pLacI | F⁻ *ompT hsdS*$_B$($r_B^-$ $m_B^-$) *gal dcm lacY1* (DE3) pLacI ($Cm^R$) |

1. The original *trxB*/*gor* double mutant (10) required reducing agent in the growth medium to support normal growth rates. The Origami and Rosetta-gami strains are a derivative (FA113) of the original strain that carry an undefined mutation which allows normal growth rates in the absence of supplemental reducing agent (13).

2. pRARE, pLysSRARE and pLacIRARE encode the tRNA genes *argU, araW, ileX, glyT, leuW, proL, metT, thrT, tyrU and thrU.*

## Genetic Marker Description

| Marker | Description | Marker | Description |
|---|---|---|---|
| *ara* | Unable to utilize arabinose. | *met* | Requires methionine for growth on minimal medium. |
| *dcm* | No methylation of cytosines in the sequence CCWGG. | *mtl* | Unable to utilize mannitol. |
| e14 | Excisable prophage element that carries the *mcrA* gene. | *ompT* | Lacks an outer membrane protease; improves recovery of intact recombinant proteins. |
| *endA* | Endonuclease I activity absent; thought to improve quality of plasmid minipreps. | P1 | Lysogenic for P1 prophage; expresses cre recombinase. BM25.8 carries an restriction minus P1 derivative. |
| F- | Strain does not contain the F episome. | pLacI | Contains a $Cm^R$ plasmid (pACYC184) that carries the gene for *lac* repressor. |
| $F^+$ | Strain contains the single copy F plasmid. | pLysE, pLysS | Contains a $Cm^R$ plasmid (pACYC184) that carries the gene for T7 lysozyme. |
| F' | Strain contains an F plasmid which harbors some bacterial chromosomal DNA. | pRARE | Contains a $Cm^R$ plasmid (pACYC184) that carries the tRNA genes for several codons rarely used in *E. coli*. |
| *gal* | Unable to utilize galactose. | pMC9 | Contains a $Tc^R$ $Ap^R$ plasmid (pBR322) that carries $lacI^q$. |
| *gor* | Abolishes glutathione reductase. | *proAB* | Requires proline for growth on minimal medium. |
| *gyr* | Mutation in DNA gyrase. Confers resistance to naladixic acid. | *recA* | Abolishes homologous recombination. |
| *hfl* | High frequency of lysogenization by phage λ. | *recD* | Abolishes ExoV exonuclease activity. Useful for propagation of sequences with inverted repeats. |
| *his* | Requires histidine for growth in minimal medium. | *relA* | "Relaxed" mutation permits RNA synthesis in the absence of protein synthesis. |
| *hsdR* | Abolishes restriction but not methylation of certain sequences ($r^- m^+$). | *rpsL* | Carries a mutation in a ribosomal protein conferring resistance to streptomycin. |
| *hsdS* | Abolishes both restriction and methylation of DNA at certain sites ($r^- m^-$). | *srl* | Unable to utilize sorbitol. |
| $\lambda imm^{434}$ | Lysogen conferring kanamycin resistance and immunity to infection by phage 434 immunity group. | *strA* | (same as *rpsL*). |
| *lac* | Unable to utilize lactose. | *supE* | Amber suppressor strain; inserts gln suppressor tRNA for UAG codon. |
| $lacI^q$ | Produces a high level of *lac* repressor. | *supF* | Amber supressor strain; inserts tyr suppressor tRNA for UAG codon; required for lytic growth of *Sam* 7 or S100 λ. |
| Δ(*lac*)U169 | Deletion of entire *lac* operon from the chromosome. | *thi* | Requires thiamine for growth in minimal medium. |
| Δ(*lac*)X74 | Deletion of entire *lac* operon from the chromosome. | Tn *5* | Contains the $kan^R$ transposable element, Tn*5*. |
| Δ(*lac-proAB*) | Deletion of the entire lac operon and neighboring genes for proline biosynthesis. | Tn *10* | Contains the $Tc^R$ transposable element, Tn*10*. |
| *lacY* | Abolishes *lac* permease. | Tn *1000* | Contains the γδ transposon. |
| *lacZΔM15* | Lacks coding region for amino terminal portion of β-galactosidase (aa 11-41). | *traD* | Defective for ability to transfer F episome DNA. |
| *lon* | Deficient for an ATP-dependent protease; thought to stabilize some foreign proteins. | *trp* | Requires tryptophan for growth in minimal medium. |
| *mcrA* | Does not restrict DNA modified by *Hpa* II methylase at $C^{me}CGG$. | *trxB* | Abolishes thioredoxin reductase. Allows formation of disulfide bonds in *E. coli* cytoplasm. |
| *mcrB* | Does not restrict DNA modified at *RmeC* by a variety of cytosine methylases. | *xyl* | Unable to utilize xylose. |
| Δ(*mcrC-mrr*) | Deletion of six genes, *mcrC, mcrB, hsdS, hsdM, hsdR, mr*r involved in methylation/restriction. | | |



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| | |
|---|---|
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| *E. coli* B834(DE3) | *E. coli* Origami B(DE3)pLysS |
| *E. coli* B834(DE3)pLysS | *E. coli* Origami B(DE3)pLacI |
| *E. coli* BL21(DE3) | *E. coli* Rosetta(DE3) |
| *E. coli* BL21(DE3)pLysS | *E. coli* Rosetta(DE3)pLysS |
| *E. coli* BL21(DE3)pLysE | *E. coli* Rosetta(DE3)pLacI |
| *E. coli* BL26(DE3)pLysE | *E. coli* Rosetta-gami(DE3) |
| *E. coli* BL21*trxB* (DE3) | *E. coli* Rosetta-gami(DE3)pLysS |
| *E. coli* BL21*trxB* (DE3)pLysS | *E. coli* Rosetta-gami(DE3)pLacI |
| *E. coli* BLR(DE3) | *E. coli* RosettaBlue(DE3) |
| *E. coli* BLR(DE3)pLysS | *E. coli* RosettaBlue(DE3)pLysS |
| *E. coli* HMS174(DE3) | *E. coli* RosettaBlue(DE3)pLacI |
| *E. coli* HMS174(DE3)pLysS | *E. coli* Tuner(DE3) |
| *E. coli* HMS174(DE3)pLysE | *E. coli* Tuner(DE3)pLysS |
| *E. coli* NovaBlue(DE3) | *E. coli* Tuner(DE3)pLacI |
| *E. coli* Origami(DE3) | Bacteriophage λCE6 |
| *E. coli* Origami(DE3)pLysS | Bacteriophage λDE3 |

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